Just under the cell membrane, so-called subpellicular microtubules could be seen (Fig. 2a). These microtubules are closely associated to the cell membrane and run in parallel with the ciliary line, suggesting that they might have a cytoskeletal role without being related to the contraction-elongation cycle.

DISCUSSION

Just like in the present organism, another heterotrichous ciliate, *Stentor coeruleus*, also shows the rapid contraction [11–13], although the style is not twisting contraction. This organism also has the similar fibrillar systems, myoneme and microtubular sheets to those in *Spirostomum*. In this *Stentor*, every myonemal filament is known to be 4 nm in diameter in the extended state. In the contracted state, however, the filaments (10 to 12 nm in diameter) appear instead of them, which are to be of tubular profiles with a wall thickness of 4 to 5 nm. In the contracted state, the wall of filaments is made up of four to six subunits.

As to the chemical nature of myoneme, Hobbs *et al.* [14] have described in *Spirostomum teres* that arrowhead decoration was not observed in cytoplasmic filament bundles although the myosin subfragment S-1 was introduced into the cells for incubation under conditions suitable for actin decoration. Furthermore, Yogosawa-Ohara and Shigenaka [2] have published in *Spirostomum ambiguum* that cytochalasin B treatment did not cause degradation of myonemal filaments even at a higher concentration (50 μg/ml). These observations strongly suggest that the myonemal filaments may not be actin-like, but may be similar to the spasmonemal filaments of peritrichous ciliates, *Vorticella* and *Carchesium* [15, 16] or the retraction fiber filaments of a dinoflagellate, *Ceratium* [17].

On the other hand, microtubular sheets which are derived from antero-posteriorly arranged ciliary pairs are overlapping and might slide relatively with each other. When contracted, the overlapping microtubular sheets increase their number in cross setions as described by Huang and Pitelka [12]. When the cell was fixed in isometric contraction, the microtubular sheets were in the state of elongation and the overlapping microtubular sheets were at minimum in number. Moreover, the internal structure of the myoneme altered in the contracted state; the myonemal filaments became to be of tubules with the diameter of 10 to 12 nm as described above.

This observation has suggested that the myoneme generates the motive force resulting into cell shortening [11]. It is thought that the myoneme and the microtubular sheets might function as antagonistic elements to each other. That is to say, the myoneme generates the motive force for cell contraction, although the microtubular sheets slide with each other to cause only cell elongation.

The present *Spirostomum* demonstrates the fine structures which are quite similar to those in *Stentor*; the myonemal filaments change their diameters and structures themselves and the microtubular sheets increase in number as seen in a cross section of organism in the contracted state. Therefore, it may be said that the myoneme and microtubular sheets of *Spirostomum* might function just like those of *Stentor*.

If the myoneme and the microtubular sheets have a function as an antagonistic system, the force of each fibrillar system must be transmitted to induce the movement of antagonistic systems of them. As the candidate for this, the rootlet-like structures might be considered to transmit the force of antagonistic system. On the other hand, the anterior fiber sheets are attached to the microtubular ribbons in the elongated state but detached from them in the contracted state, so they may have a function as the "trigger" or "switch" for inducing contraction and/or elongation of the cell body.

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Gonadal Maturation Independent of Photic Conditions in Laboratory-Reared Sea Urchins, *Pseudocentrotus depressus* and *Hemicentrotus pulcherrimus*

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ABSTRACT—The gametogenesis in two species of the laboratory-reared sea urchins, *Pseudocentrotus depressus* and *Hemicentrotus pulcherrimus*, were compared under different artificial photic conditions. Juvenile sea urchins derived from zygotes of the same batch were reared under constant illumination at 20°C until they were transferred to experimental environments, where they were kept for about a year. The experimental environments were: continuous light at constant temperature (20°C) (20 LL); continuous light at ambient temperatures (Amb LL); continuous darkness at ambient temperatures (Amb DD); changing photoperiod in phase with ambient daily photoperiod at 20°C (In-phase); changing photoperiod 6 months out of phase with ambient daily photoperiod at 20°C (Out-of-phase); and ambient light at ambient temperatures (Control). The stages in gametogenesis were determined according to a histological standard.

In *P. depressus*, the gonads in the ambient temperature groups (Amb DD, Control) matured simultaneously; the gonads in the constant temperature groups (20 LL, In-phase, Out-of-phase) also became ripe at the same time, but earlier than those in ambient temperature groups (Amb LL was lost by an accident). In *H. pulcherrimus*, the gonads in ambient temperature groups (Amb LL, Amb DD, Control) ripened simultaneously; but in the groups kept at 20°C (20 LL, In-phase, Out-of-phase), the gonads remained very immature. These results indicate that in the two species no specific photic conditions are required for gametogenesis to proceed. The results in *H. pulcherrimus* also suggest the presence of a critical temperature to permit the progression of gametogenesis.

INTRODUCTION

Sea urchins in shallow waters of the Temporal Zone generally have a specific breeding season. The annual reproductive cycles have been studied in detail in many sea urchin species [1–11]. In these studies, the environmental factors controlling the reproductive phenomena are evaluated from links between reproductive cycles and environmental fluctuations, or from comparisons between populations of the same species collected from different localities — photoperiods, temperatures, foods and the lunar cycle have been presumed to serve as seasonal cues to synchronize the reproductive cycles. There are, however, a few data on the gonadal response to experimentally manipulated environmental factors. To our knowledge, such studies have been done only in *Strongylocentrotus purpuratus* collected from the west coast of North America [12–16]. Recently Pearse *et al.* [15, 16] have experimentally demonstrated in *S. purpuratus* that growth and gametogenesis are under photoperiodic control.

We have started a series of long-term experiments on environmental control of gametogenesis in Japanese sea urchins. We used animals reared from fertilized eggs under a constant artificial condition in the laboratory so that they are as free as possible from the influence of the natural environment. In contrast to the Pacific coast of North America, where the seasonal change in sea temperature is moderate, it is marked along the coast of Japan. It is thus conceivable that Japanese sea urchins are under different environmental control from the *S. purpuratus*. In this paper, we

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present experimental evidence showing that photoperiod is not at least a main environmental cue to synchronize gametogenesis in two common Japanese sea urchins, *Pseudocentrotus depressus* and *Hemicentrotus pulcherrimus*.

MATERIALS AND METHODS

Adult sea urchins

Adults of *Pseudocentrotus depressus* for obtaining gametes were collected from the subtidal zone near the Gaikai Fishery Experimental Station of Yamaguchi Prefecture in November 1985. The breeding season of this species there is from October to December. Adults of *Hemicentrotus pulcherrimus* for obtaining gametes were collected in the intertidal zone near the Ushimado Marine Laboratory, Okayama Prefecture, in March 1986. The breeding season of this species there is from January to March.

Rearing of juvenile sea urchins

The experiments were carried out in the Ushimado Marine Laboratory. Embryos were obtained by artificial fertilization of gametes spawned from one male and female on 26 November 1985 in *P. depressus* and on 29 March 1986 in *H. pulcherrimus*. Larvae and juvenile sea urchins were reared according to the method by Kakuda [17–19] after slight modifications. The embryos were kept in 30 liter tanks (600 embryos/liter) at 20°C and fed with diatom *Chaetoceros gracilis* that was cultured in the laboratory. When the echinus rudiment became prominent in 8-armed pluteus larvae (21 day and 15 day after fertilization in *P. depressus* and *H. pulcherrimus*, respectively), metamorphosis was induced by giving plastic plates on which a film of diatoms had been prepared by soaking the plates in running sea water for several days. The plastic plates with metamorphosed juvenile sea urchins were transferred into a 500 liter tank provided continuously with small amount of running sea water. The tank was kept in a water bath at 20°C under continuous illumination of the ceiling lights. When the test of the juvenile sea urchins reached about 1 mm in diameter, the sea urchins began to be fed with sea lettuce, *Ulva pertusa*. When the test diameters became about 7 to 12 mm in *P. depressus* (18 March 1986) and about 4 to 7 mm in *H. pulcherrimus* (21 June 1986), juvenile sea urchins were divided into 7 groups of 70–80 individuals that were matched in size distribution and transferred into experimental environments.

Experimental environments

Individuals in each group were placed in a round plastic cage, 30 cm in diameter, and kept under one of the following experimental environments:

Group 1 (20 LL); constant temperature (20°C) and continuous light.
Group 2 (20 DD); constant temperature (20°C) and continuous darkness.
Group 3 (In-phase); constant temperature (20°C) and in-phase photoperiod regime (see below).
Group 4 (Out-of-phase); constant temperature (20°C) and out-of-phase photoperiod regime (see below).
Group 5 (Amb LL); ambient temperature and continuous light.
Group 6 (Amb DD); ambient temperature and continuous darkness.
Group 7 (Control); ambient temperature and ambient light.

For maintaining each of Groups 1–6, we used separate indoor aquaria ($120\times65\times45$ cm), each equipped with a system to recirculate constant temperature sea water. In the aquaria for constant temperature groups (Groups 1–4), the recirculating sea water kept at 20 ± 1°C was continuously replaced little by little by new sea water. The aquaria for ambient temperature groups (Groups 5 and 6) were continuously supplied with running sea water at ambient temperatures. The illumination for continuous light in Groups 1 and 5 was given by some 40 W fluorescent bulbs (about 2000 lux). The aquaria for continuous darkness and photoperiodic regimes (Groups 2, 3, 4 and 6) were made light-tight using opaque plastic boards. The illumination for photoperiodic regimes (Groups 3 and 4) was given by a 40 W waterproof fluorescent bulb equipped inside each aquarium and control-

led by a time switch outside. In the in-phase photoperiod regime, the light went on at local sunrise and off at local sunset. In the out-of-phase photoperiod regime, the light went on and off at the time when the light in the in-phase aquarium went off and on, respectively, thus the photoperiod being set to be 6 months out of phase with the ambient daily photoperiod. The time switches were reset every 7 days according to the changes in the ambient daily photoperiod. The control group (Group 7) was kept in an outdoor tank supplied continuously with running sea water at ambient temperatures.

The sea urchins were provided with an unlimited amount of food. They were fed with *Ulva pertusa* from March to October and some species of *Sargassum* (*S. horneri, S. serratifolium, S. tortile* etc.) from November to February. Feces and sediments were removed from the aquaria once a week. The lid of the aquaria for the continuous dark groups (Groups 2 and 6) was opened briefly (less than 15 min) every 3 or 4 days at an unfixed time for feeding and cleaning.

Sampling

The test diameters and wet weights of all animals were periodically measured. Some randomly selected animals from each group were periodically dissected for histological analysis. The gonads were weighed and pieces were fixed in Bouin's solution. The paraffin-embedded gonadal tissues were sectioned and stained in Papanicolous' haematoxylin and eosin. The experiments were continued until all individuals were sampled.

RESULTS

Stages in gonadal development

The gonad index (percentage of wet gonad weight in wet animal weight) is often used as an indication of the gonadal maturation, but we think it is unsuitable to the present experiments; not only exact measuring of wet gonad weights in small sea urchins is difficult but the gonad indices do not fully correlate with gonadal maturities because of

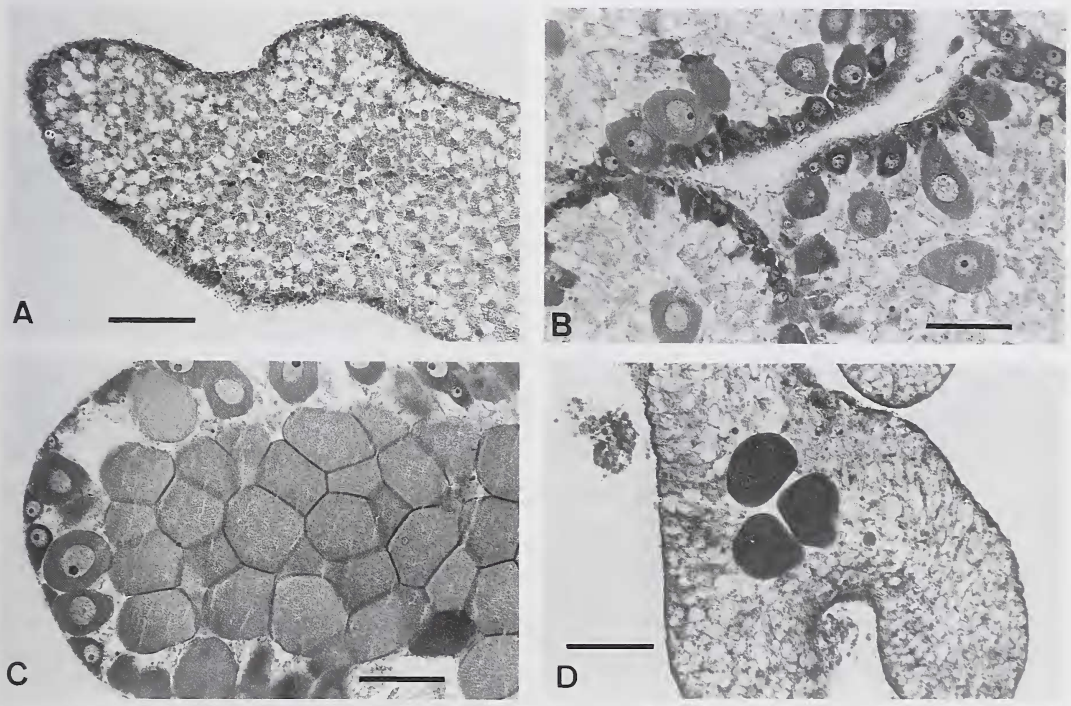

FIG. 1. Sections of the ovary in *Hemicentrotus pulcherrimus*. a, Stage 1; b, Stage 2; c, Stage 3; d, Stage 4. Scale, 100 μm.

rapid growths of sea urchins themselves. In the present study we found it most suitable to determine the stages of gonadal development according to a morphological standard defined in advance.

Previous authors have divided the process of gonadal maturation in sea urchins into 4 to 9 stages [2, 4, 10, 11]. After a survey of many histological sections, we defined the following 5 stages in development of the sea urchin gonad.

Stage 0: No obvious germ cells are found; sexes of the gonad cannot be identified in the section.

Stage 1: *Ovary* — A few small oocytes are present along the periphery of the ovarian wall. No large oocytes were found (Fig. 1a).
Testis — Small clusters of spermatogenic cells are present along the periphery of the testicular wall (Fig. 2a).

Stage 2: *Ovary* — Many large oocytes with a prominent germinal vesicle are present in the ovarian wall. Some oocytes migrate toward the center of the ovarian lobe (ovarian cavity). No mature eggs are found (Fig. 1b).
Testis — The wall of the testicular lobe is lined with columns of spermatocytes. Small masses of spermatozoa are present in the center of the testicular lobe (lumen) (Fig. 2b).

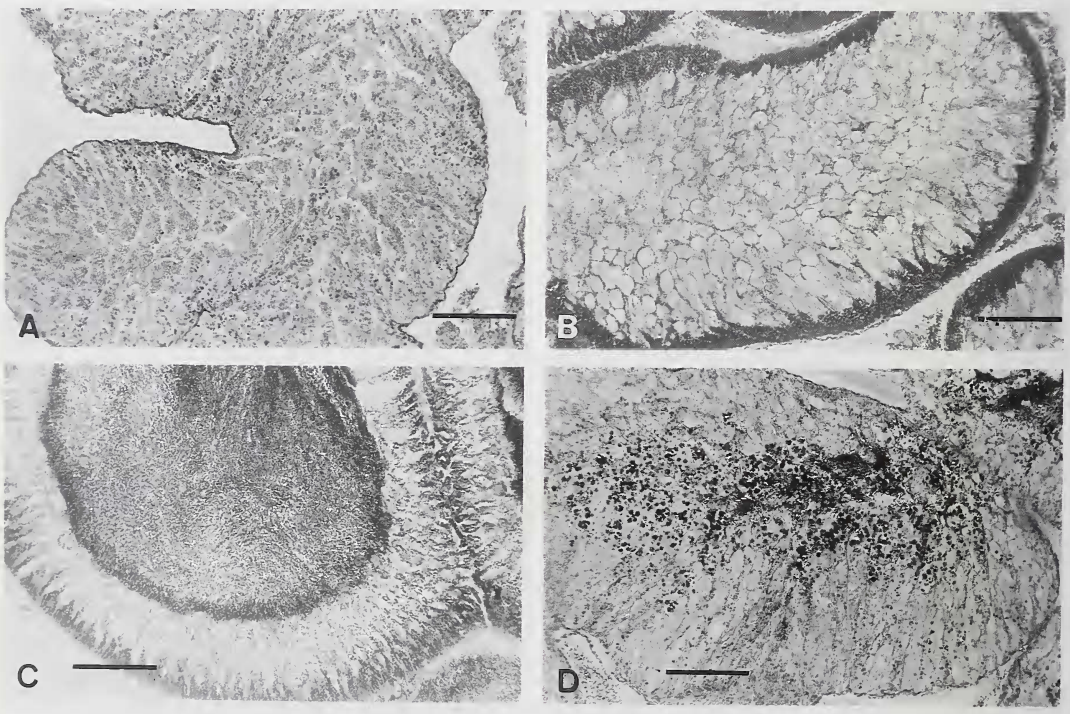

Fig. 2. Sections of the testis in *Hemicentrotus pulcherrimus*. a, Stage 1; b, Stage 2; c, Stage 3; d, Stage 4. Scale, 100 μm.

Fig. 3. The stages of the gonads of *Pseudocentrotus depressus* maintained under 5 experimental environments. Each symbol represents one individual. Sea urchins were reared from zygotes of the same batch fertilized on 26 November 1985 and were kept under constant illumination at 20°C until they were transferred to one of the following experimental environments on 18 March 1986: continuous light at 20°C (20 LL); changing photoperiod in phase with ambient daily photoperiod at 20°C (In-phase); changing photoperiod 6 months out of phase with ambient daily photoperiod at 20°C (Out-of-phase); continuous darkness at ambient temperatures (Amb DD); ambient light at ambient temperatures (Control). Sea water temperature near the Ushimado Marine Laboratory is shown at the top.